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DEVELOPMENT OF AN ELECTRODE FOR LONG TERM APPLICATION IN BIOLOGICAL RECORDING

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(Final Rept.)

Oct. 1963

Prepared under Research Contract NAS-9-445 between NASA Manned Spacecraft Center and Baylor University College of Medicine.

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FOREWORD

This investigation was conducted under NASA Research Contract NAS 9-445 with Baylor University College of Medicine. It was inspired as a result of a Conference on Bioinstrumentation held at the Manned Spacecraft Center on January 18-19, 1962. Many of the ideas for this approach to the electrode problem originated with NASA personnel present at that conference. The work was accomplished at the Houston State Psychiatric Institute for Research and Training. The principal investigator is grateful for the cooperation and many helpful suggestions by Mr. Maxwell W. Lippitt, Jr., NASA Technical Director for this contract, and for the technical assistance of Mr. Louis Galvan, Mrs. Maureen Praver and Mr. Kurt Solis.

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I INTRODUCTION

The monitoring of the physiological or psychophysiological state of a human over an extended period, either in an orbiting satellite or in long-term isolation experiments in the laboratory demands a critical link between the biological organism and the electronic system into which it is feeding. This link, namely the electrode, must satisfy the requirements of both systems, that is:

(1) It must be non-irritating and should not promote infection. (2) It should be comfortable. (3) The electrode resistance should be low, less than 10,000 ohm cm^2. (4) It should not develop any electrode bias, that is, battery effect. (5) It should not polarize during the passage of current. (6) The attachment must be mechanically strong and not susceptible to movement of the attached leads. (7) There should be no contact of two

dissimilar metals with the electrode medium. (8) The preparation should not show progressive deterioration in conductivity.

The most challenging of the above requirements is that of low resistance which does not increase over time. This investigation was designed to fulfill this requirement as its prime objective while at the same time giving attention to the others.

The approach to this problem was guided by the following considerations. Intact skin under an electrode will offer two primary sources of resistance. It has been shown that at least 80% of wet skin resistance resides in a thin layer of the epidermis, most likely the stratum lucidum (8), which shall be referred to as the <u>living barrier layer</u>. The resistance of this layer may be artificially reduced by the application of certain chemicals (2). If the skin is wet, even with a dilute electrolyte, the <u>second</u> primary source of its resistance, the stratum corneum, does not constitute an effective source of resistance. The resistance of the living barrier layer will range between 5000 and 500,000 ohms cm^2 depending upon where on the body it is measured and upon the condition of the subject. Dry corneum, however, may contribute more than a megohm cm^2 to the total resistance.

Two approaches to the solution of this problem present themselves: (a) to reduce the resistance of the corneum either by abrasion, by keeping it wet, or by converting it to a dry conductor and (b) to artificially reduce the resistance of the underlying cellular layer.

II CONVERSION OF THE CORNEUM TO A DRY CONDUCTOR

A. THEORETICAL APPROACH

Conversion of the corneum to a dry conductor essentially entails the deposition of a free metal within the loose keratin network which becomes a supporting stroma for the infiltrated mass of metal. Since this stroma is too densely packed to allow the infiltration with metallic particles, even of colloidal size, a feasible method of accomplishing this end would be to infiltrate with a metallic ion which would be subsequently reduced. If upon subsequent reduction the metallic particles remain discreet and dispersed throughout the stroma, conductivity would be improved only if the space between particles were slightly moist and if the current flow in part passed through these isolated particles. If on the other hand the precipitated mass of reduced metal formed a continuous network, conductivity would occur regardless of the degree of hydration of the corneum.

Early experiments on model systems consisting of particles of metal distributed discreetly in a conducting electrolyte demonstrated that these particles did enhance the conductivity of the mass and that they in effect became dipoles, one end becoming anodized, the other cathodized. Although this laid the groundwork for assuming that even a dispersion of discreet particles within the corneum would improve conductivity, experiments of this sort were shortly discontinued when it became evident that a stroma of cellulose or protein, infiltrated with a silver salt, could be converted to a dry continuous conductor having stable properties. This development, together with the evidence for the existence of a continuous conducting structure are discussed in this section.

B. INFILTRATION OF FILTER PAPER

1. Method

Strips of filter paper 1x6 cm. were exposed to a silver nitrate solution, then to light, and subsequently to a reducing solution. After reduction the strips were washed thoroughly in tap water, then in distilled water and finally dried. Resistance measurements were made at times with the strips sandwiched between two plates, 1 cm^2, and at other times down the length of the strip between two plates 5 cm. apart. Details of secondary procedures will be discussed in the relevant section.

2. Results

Preliminary efforts were made to use the sebacic acid salt of silver so that the product released as a result of reduction would be a weak organic acid of a type naturally occurring in the skin. Because of the slight solubility of this material efforts to reduce it even after exposure to strong ultra-violet light for several minutes failed to produce a conducting mass within the filter paper. Silver nitrate proved to be a highly satisfactory agent and was adopted as the standard salt. Parameters of the infiltration method which were investigated consisted of concentrations of silver nitrate, intensity of light, nature of reducing agent and times of exposure to each of these parameters. The results may be summarized as follows: Silver nitrate at 0.5 M concentration was adequate to produce a highly conducting infiltration. Exposure of the filter paper to silver nitrate for only 10 seconds was as effective as much longer exposure times. An exposure time of 10 seconds to light at a distance of 12 inches from a 100 watt bulb proved to be as effective as significantly longer periods. Light from a 100 watt bulb was as effective as ultra-violet light. Standard X-ray developing solution proved to be a very effective reducing agent. Fresh ascorbic acid solution at a concentration of 0.1 M proved to be almost as rapid for development as was the X-ray developer. Filter paper strips 1cm. by 5 cm. showed resistance ranging from 5 to 200 ohms from end to end and less than 2 ohms across the thickness ($1cm^2$). Control strips treated exactly the same but with NaCl substituted for the $AgNO_3$ showed resistances greater than 20 megohms across the thickness.

C. INFILTRATION OF OTHER MATERIALS

The optimum infiltration procedure was applied to various other materials including cellulose sponge, plastic sponge, hair, leather, and gauze. Highly conducting infiltrations were achieved in all cases except on plastic sponges and other plastic materials. Leather infiltration was successful but required longer times of exposure because of the problem of diffusion and required longer exposure to light probably because of the optical density. Both the leather and human hair were converted from a pliable to a stiff material with hair becoming especially brittle in the process.

D. NATURE OF THE INFILTRATION

Since the filter paper strips had been washed thoroughly in distilled water, it was unlikely that salt was contributing to the conductivity, especially in view of the negative results with the sodium chloride controls. Furthermore the conductivity of these filter paper strips was just as high when measured during continuous drying in a hot air blast as when allowed to reach equilibrium with ambient humidity and it was concluded that metallic silver was responsible for the high conductivities.

Other evidence pointed to the fact that the infiltration produced a continuous threadlike silver network rather than a cluster of particles in contact. For one thing the silver strips did not behave like carbon microphones, resistance changing only slightly during flexing of the strip. Folding the strip in half produced a distinct rise in resistance as might have occurred if the continuous threads had been broken at this point. Furthermore, microscopic examination of the unsuccessful deposition on plastic showed that the particles were aggregated on these surfaces into discreet, non-continuous masses, whereas examination on the successful infiltrations, whether on paper, hair, or leather showed no evidence of particles; the appearance was as if the structure of the original material had been stained. It was tentatively concluded that in these successful cases there is an initial attraction between the silver and the substrate such that an intimate physical relation is initially produced and endures even after reduction of the silver. Further evidence on this point will be discussed in a subsequent section relating to the study of the ultra-structure of the infiltrated mass. A last piece of evidence in favor of a continuous network rather than a close assemblage of particles is the effect of impregnation of the silver network with molten paraffin or Pliobond cement. When a mass of finely divided silver or carbon is infiltrated with either of these agents, the original high conductivity of the mass disappears entirely and it becomes an effective insulator. It is presumed that the particles are surrounded and separately insulated by infiltration with the liquid material. However, when a strip of filter paper was exposed to molten paraffin in such a way that the ends were left free to make electrical contact, the conductivity was unaffected, the only change being that the strip became less susceptible to the effects of flexing and bending. When these strips were exposed to Pliobond in a closed container to enable the Pliobond to remain liquid long enough for infiltration to take place, the same results were achieved. It was concluded from these experiments that the silver elements of the network were laid down in such a continuous and stable array that molten paraffin or Pliobond cement could not diffuse between the particles to produce an insulating effect.

E. INFILTRATION OF HUMAN SKIN

1. Method

The basic procedure adopted for filter paper and leather was used in studies on humans. The skin was washed clean of its chloride, first in tap water and then in distilled water, air dried and subjected to the procedure discussed above. Following infiltration, resistances were measured immediately after drying with a warm air blower. A probe of constant force and area was lowered in turn on each silver site as well as on the skin sites. The circuit was completed by dipping two fingers into a beaker of saline containing the opposite silver electrode. The influence of variation in each parameter was investigated in a fashion similar to that used with the filter paper but because of the variability in the skin from one time to another and from subject to subject, a special arrangement had to be made so that anatomical area and temporal conditions would be cancelled out in the comparison.

The procedure was as follows: Six to eight sites, each two mm. in diameter arranged in a double row with a spacing of 5 to 10 mm. between sites were ordinarily set up. In later experiments sites were 6 mm. in diameter. A given condition was tested in duplicate or triplicate in this arrangement and from two to four variables were examined at the same time. When the time variable was under examination each site served for a different duration. Although resistances were initially measured with reference to the fingers in saline, it became clear that the unknown composition of the medium in contact with the silver network at the basal region of the epidermis frequently gave rise to a bias potential between the reference electrode and the test electrode and introduced an error in the resistance reading. Because of this, later measurements were made between two identically prepared sites. A wet electrode, prepared in a conventional manner with 0.05 M NaCl - starch paste (1), was used to give an indication of the contribution of the underlying layer to the total resistance. Noise level data on these preparations was obtained by taping a metal plate to two of these sites and recording into a DC Offner amplifier having a sensitivity of 1 microvolt per millimeter.

Electrode bias was measured during these noise level recordings.

2. Results

The immediate effects of the various parameters are best presented in tabular form (table 1). The overall conclusions may be summarized as follows: (1) An acetone rub prior to infiltration improves conductivities by about 100 per cent. (2) Skin drilling by the method of Shackel (11), modified such that only the upper layer of corneum is removed, commonly improves infiltration by about 100 per cent, although in some instances there is no improvement. The use of skin stripping by the adhesive tape method of Wolf (12) and Pinkus (7), or the use of fine sandpaper, has essentially the same effect. (3) There is essentially no difference whether silver nitrate is applied to dry skin or to pre-soaked skin. (4) The minimum exposure time of any subject to silver nitrate in order to achieve optimum results is five minutes (figure 1). The minimum development time is two minutes. Development with 0. 5 M ascorbic acid is as effective as with X-ray developer. (5) The application of an anodal current (produced by a 6 volt source) to accelerate diffusion of silver ions by iontophoresis improves results by about 30% but in some subjects results in a burn. Reduction of the voltage to 3 volts did not preclude irritation or injury in some subjects, though the effects were ameliorated. The rate of acceleration of silver penetration at 6 volts or less is insufficient to justify risking skin damage and the use of iontophoresis has

TABLE 1

Effect of various procedures before and during silver infiltration of human skin. Values are initial dry resistance readings on an area of 0.31 cm^2.

	Resistance (Megohms)								
Subject	1	2	3	4	5	6	7	8	9
Condition									
No infiltration	>10	>10	>10	>10	>10	>10	>10	>10	>10
Starch-NaCl	.13	.9	.7	.6	1.5	.25	.13	.50	
Ag-Infiltrated	.23	>10	.6	.7	4.0	.60	.08	.04	
$AgNO_3$ exposure 5 min.	.90	.64	.55	.49	2.5	2.8	5.8	.5	1.2
16 min.	.50	.22	.50	.14	.5	.2	1.3	.4	0.1
No acetone pre-wash	.70	2.5	3.2	1.20	1.0				
Acetone pre-wash	.30	1.2	5.2	.60	.04				
Pre-soaked in H_2O 10 min.	.44	1.0	10+	5.0					
Pre-soaked in 1% Tergitol (detergent) 10 min.	2.0	6.0	9.0	5.0					
Not pre-soaked	.28	.31	.24	1.90	.19	.33			
Pre-soaked in H_2O 10 min.	.34	.24	.18	.58	.15	.55			
$AgNO_3$ used pure	.75	.07	.04	7.1	.19	.008	1.59		
Ag^+ converted to $Ag(NH_3)^+$	1.38	.30	2.33	8.0	.53	.020	.60		
Pre-soaked in H_2O 15 min.	.40	.34							
Pre-soaked in 0.05 NaCl 15 min.	.75	.65							
Single infiltration of Ag^+ 20 min. duration	.39	.13	.72						
Single infiltration of Ag^+ 10 min. duration	.52	.20	5.6						
Second 10 min. infiltration on top of first	.40	.08	.26						

TABLE 1 (cont'd)

	Resistance (Megohms)									
	1	2	3	4	5	6	7	8	9	
Not abraded	1.17	1.65	.80	2.5	.75	3.17	.53	.75	.15	
Abraded	.07	.58	.42	.08	1.60	1.40	.54	.09	.12	
0.1 M Ascorbic Acid	.57	2.4	.33	.60	.63	2.40)*	Note 1 & 2, 3& 4			
0.2 M " "	.10	1.37	.43	2.7	2.70	1.45)	5&6 are pairs of			
1.0 M " "	.57	.80	.40	.70	1.36	4.20)	sites on three subjects			
X-ray developer	.70	.87	.056							
0.1 M Ascorbic acid	.52.	.22	.067							
No iontophoresis	1.00	.80	1.53	.85	1.20	>20	3.0	>20		
Iontophoresis (12-22v.)	.49	.58	1.28	.50	.73	1.70	.30	.13		
No skin-stripping	2.05	1.77								
Skin-stripping by Wolf tape technique	1.53	1.28								

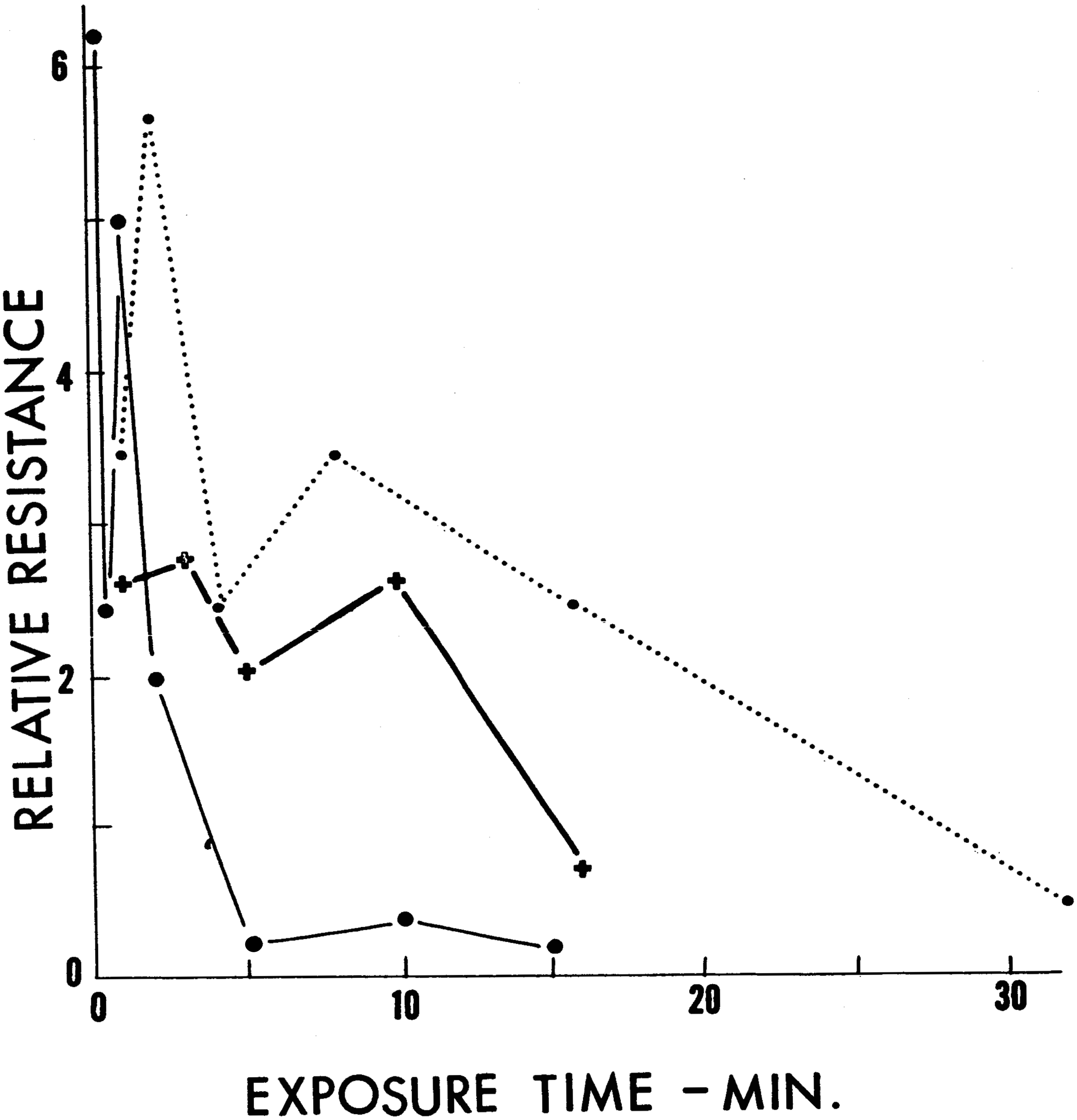

Figure 1. - Effect of duration of exposure to $AgNO_3$ upon final resistance of dry site. Curves on 3 out of 8 subjects are illustrated. Each point represents a separate site. Variability reflects differences between sites rather than uncertainty of readings.

been abandoned in favor of longer soaking times. (6) Sites on hairy areas became more conductive than sites on non-hairy areas. This may have favorable implications with regard to the use of the method for EEG. (7) Two separate infiltrations proved to be as effective cumulatively as a single infiltration of equal total duration. (8) Alteration of acidity from pH 5 to pH 9 interferes with the success of the infiltration in most cases. (In order not to precipitate out the Ag^+ in the alkaline medium conversion of $AgNO_3$ to the $AgNH_3^+$ complex before infiltration was necessary). (9) Electrode bias between two infiltrated sites is negligible. (10) Noise level with this method of electrical contact is unacceptably high.

A program of routine testing using the comparison technique led to the selection of an optimum processing schedule as outlined below.

a. Wash site in soap and water and rinse thoroughly. Dry and rub with acetone moistened gauze.

b. Rub site lightly with fine sandpaper to remove only the surface layer.

c. Apply masking tape with 1 cm. square hole cut out.

d. Apply puddle of 0.5 M $AgNO_3$: soak 25 minutes.

e. Dab off excess $AgNO_3$ and expose to 60 watt bulb at a distance of one foot for 5 minutes.

f. Apply puddle of 0.5 M ascorbic acid and let act for 10 minutes.

g. Wash in tap water for one minute.

h. Remove mask and dry site under warm (not hot) air blower.

Using the optimum method described above, the conductance of the freshly infiltrated dry site was routinely as high as that of the control wet site. However, when followed for several days the conductivity of the sites was found to deteriorate at a very variable rate, many subjects showing an almost complete loss of conductivity within a space of hours, while a few maintained high conductivity even after four to six days. Subsequent experiments have been run in which long term monitoring of the site was accomplished in the presence of a "skin solder" electrical contact to be described below. The results of these tests undertaken with the site exposed to the atmosphere are summarized in table 2. It should be noted that the black discoloration caused by the infiltration became noticeably lighter in two to three days and in most cases had eroded away within a week. In some cases, however, the depth of penetration was so great that even after a week the black discoloration was still intense. This observation lead to the conclusion that one of the critical variables in the preparation would be the depth of penetration and that procedures which facilitate diffusion in the corneum should enhance the success of the preparation. As will be discussed subsequently, however, depth of penetration is not the only key variable; maintenance of the silver in a reduced form proves to be just as important.

TABLE 2

Daily resistance of uncovered silver-infiltrated human skin in proportion to value on day of infiltration (i. e. R_t/R_o). Values in a given row are for the same site.

DAYS OLD 0	1	2	3	4	5
RESISTANCE RATIO					
1				1.25	.82
1	1.20				8.0
1	.96				
1	.84				
1	.10				
1	2.03			>72.	
1	1.57			>59.	
1	.19				
1	.81				
1	1.96			>65.	
1	2.70			>71.	
1	.57	.60			
1		13.4			
1		>83.			
1	>12.2				
1	1.63				
1	1.25				
1	1.53				
1	1.09				
1			1.15		
1			2.30		
1	1.30				
1			18.7		
1	>10.				
1	.95				
1	>2.5				
1	8.7				
1			1.00		
1			1.92		
1			2.50		
1		1.25			

The noise level was relatively high when measured as described under "Method" and was never found to be less than 20 microvolts at this stage of the investigation. Previous experience suggested that the source of this noise was at the junction between the infiltrated site and the metal electrode. This seemed especially plausible since the noise level between two infiltrated filter paper strips dipping into saline was of the order of one to two microvolts, similarly for leather.

In these experiments electrical contact was made with an alligator clip firmly attached to the dry end. On the basis of this observation, efforts were directed toward development of a stable electrical connection to the site as will be discussed in the next section.

III DEVELOPMENT OF AN ADEQUATE ELECTRICAL CONTACT WITH THE INFILTRATED SITE

Measurements of noise level between two infiltrated sites making contact with a metal plate for electrical connection had indicated the need for a special means of making electrical contact with the site since this junction was suspected as the most likely source of noise. The use of a paste to make electrical contact between the plate and the site would have the same disadvantages as a wet site. Efforts were directed toward production of a conducting cement which would bond equally well to skin and to a metal connector.

A. SELECTION OF THE CONTACT AGENT

Available conducting cements were unacceptable either because of (a) toxicity, (b) failure to bond to skin, (c) inadequate conductivity, (d) tendency to fragment when flexed. It was decided to develop a suitable conducting cement by dispersing either graphite or a metallic substance such as silver in an available cement substance. Several possibilities were eliminated on the basis of the objections outlined above and finally polyvinylpyrrolidone (PVP, General Analine Corp.) was chosen. This substance had been used during the war as a plasma expander and was clearly non-toxic. It had some bacteriostatic properties, was a protective colloid and therefore enhanced wetting of surfaces, had both hydrophylic and hydrophobic groupings and could therefore stick to polar and non-polar compounds, was water-soluble, and relatively strong when dry. Furthermore, unlike numerous other cements, being water-soluble it would not tend to become loosened if the underlying skin became moist due to sweating. The development of a suitable "skin solder" with the use of this compound is described in this section.

1. Method

Four characteristics of the sample products were tested: (a) tenacity, (b) flexibility, (c) conductivity, (d) noise level. To test conductivity and flexibility a calibrated smear of the material 7 mm. wide and 1 mm thick (when wet) was spread on typing paper. For conductivity measures two wires, set in place while the sample was still wet, were oriented transversely across this strip, 10 mm apart and taped to the paper. For the flexibility test the dry film was folded transversely, in a hinged device under a fixed pressure. The film was bent once, then unfolded and examined under a stereoscopic microscope at 10x. Results were graded in terms of susceptibility to cracking as follows:

0 - no crack in film

1 - incipient crack

2 - partially cracked

3 - completely cracked

For more refined separation of films in the 0 - 1 range, two or more cycles of the folding and unfolding process were applied. For each test two films were prepared and three samples of each were run through the flexing test with highly reproducible results. Initial tenacity tests involved measurement of the tension required to pull two pieces of paper apart when cemented with a given PVP product. However, it soon became clear that measurements of tenacity to paper may bear very little relation to the tenacity to skin and these were consequently abandoned. The tenacity to skin was very qualitatively gauged by the degree to which the edges of the cement film curled up and by the degree to which the cement fragmented away from the skin over the course of time. The noise level of the same smear used to check resistance was measured on an Offner DC microvolt recorder. When tried on humans, noise was measured between two infiltrated sites having coiled wire strands affixed with the PVP cement. Measurements were made with undisturbed electrodes and also with a calibrated disturbance. The disturbance was achieved by dropping a guided brass rod (1/4 inch diameter, 1-1/2 inches long) onto the site, end first, from a standard height (2-1/2 inches).

2. Results

Since a given mixture of PVP and water dries until it reaches equilibrium with the atmosphere, the final product is relatively independent of the concentration of the initial dispersion. The initial concentration, however, is very important with regard to ease of applying the cement. It was found that a 25% solution by weight, that is, 1 part of PVP to 3 parts of water, gave a mixture which was not so tacky that it could not be handled, which could flow into the microscopic crevices of the skin, which was still thick enough so that it did not take unduly long to harden and which was mechanically strong enough after a short period of drying to hold the metallic connector in place. The addition of various amounts of powdered graphite gave films which at low graphite concentrations, were strong and relatively flexible but of low conductivity, and. at high concentration of graphite, gave excellent conductivity but tended to become brittle and weak. A range of graphite concentrations was examined until the optimum combination of strength and conductivity was achieved (table 3). This mixture is made as follows: 2.4 parts by weight of powdered graphite (particle size approximately 0.015 mm.) to one part by weight of PVP - K90 constitutes the dry "mix". This "mix" is added to an equal weight of distilled water and after stirring, is allowed to dissolve for 24 hours in a stoppered container. Following this it is stirred thoroughly and ready for use.

Proceeding on the assumption that volume relations in the dry product, rather than mass, were critical, the dry ratio tried for silver was 11 silver: 1 PVP, to allow for its greater density. This ratio, however, gave too brittle a film. Reduction of the proportion of silver to 4.8 to 1 still gave adequate conductivity and this ratio was adopted. In practice 1.2 gm. of silver is mixed with 1 gm. of the 25% PVP solution.

In tests on humans, the infiltrated site is first prepared and dried as usual. It is next covered with a layer of skin solder about 1 mm. thick; a coiled strand of #30 silver wire is applied and covered over with more cement (about 1 mm. thick). A warm air current hastens drying which takes about ten minutes. The strand of wire may be left short (preferable for this development phase) or attached beforehand to a light flexible lead. The use of a patch of conducting Velcro cloth has shown excellent possibilities for practical application. It is cemented to the site with skin solder and later electrical contact is made by applying the partner patch.

TABLE 3

Effect of concentration of graphite and of silver on resistance of PVP strips.

Proportion by weight (Dry)	Resistance	Characteristics
Graphite: PVP		
2 : 1	>20 megohms	Strong, tenacious
2.4 : 1	155 ohms	
2.8 : 1	100 ohms	Increasingly
3.3 : 1	95 ohms	
3.6 : 1	200 ohms	brittle
4 : 1	150 ohms	Cracks, fragments
Silver : PVP		
2 : 1	1 ohm	Strong, tenacious
4.8 : 1	1 ohm	less flexible

While far superior in terms of noise level to other methods of bonding the electrical lead to skin, these preparations still show a significant and variable degree of noise, e.g. 4 to 20 microvolts undisturbed, and more than 500 microvolts when tapped gently. Further, when dry these films tended to fragment and break away from the skin. The tenacity of the film of skin solder apparently depended to a great extent on its flexibility. Flexing of the skin tended to cause fragmentation of brittle films and an associated increase in resistance. Microscopic examination of such films in situ indicated that sections had been loosened from the skin during the fragmentation process. The tearing force presumably being generated by the flexing of a brittle sheet. When thinner films, which are more flexible, undergo the same degree of flexing, the bond is significantly less disturbed. Since more flexible films give promise of lower resistance and lower noise level, efforts were directed toward increasing the flexibility of the skin solder.

B. INCREASING THE FLEXIBILITY OF "SKIN SOLDER"

Two approaches were made to the problem of increasing the flexibility of these PVP films. The first approach was based on Mosier's suggestion (5) that the PVP molecule could be plasticized by combining it with a compound which altered the ultra-structure of the gel in such a way as to facilitate greater slippage between molecules, hence greater flexibility. An assortment of agents consisting of tricresyl phosphate, triphenyl phosphate, di-iso octyl phthalide, dibutyl sebacate plus commercial plasticers of polyvinyl plastics such as Flexol and Santicizer were tested in varying concentrations all with negative results. Considerably later in the investigation the product Aquaflex* suggested by Mosier as being more suitable for plasticizing a hydrophyllic molecule like PVP, was tested with excellent results.

During the course of the above tests it was found that in many instances flexibility was increased by the additive but the simultaneous increase in the tackiness of the film lead to the belief that the agent, acting hygroscopically, was increasing the moisture content of the PVP and was essentially increasing its flexibility not by the mechanism proposed by Mosier but simply by adding more water to the mixture, in a sense by reversing the drying process. This observation lead to a consideration of a series of alcohols which were compatible with PVP and whose presence could be expected to exert a hygroscopic effect. If the alcohols were producing their increase in flexibility by a direct action on the molecular bonding of the PVP molecule it would be expected that humidity should not constitute an important factor. When various samples of PVP-alcohol mixtures including ethanol, Methanol, isopropyl alcohol,

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* Aquaflex 350 (tetrahydrofurfuryl phosphate), FMC Corp., 633 3rd Ave., New York 17, N.Y.

N-butanol, and diethylene glycol were heated in an oven to 80 degrees C. for 90 minutes to drive off the water, they were found to be essentially as brittle as ordinary samples prepared without the alcohol. These same alcohol samples, when exposed to humid room air for some time, would increase their flexibility. An examination of the increase in flexibility as a function of the nature of the added alcohol showed that the effect increased as the vapor pressure of the alcohol decreased. Glycerol, a non-toxic, hydrophyllic alcohol with a low vapor pressure, was chosen as a promising flexing agent. PVP samples made up as usual, but with various concentrations (1 to 75%) of glycerol substituted for the water, showed a progressive increase in flexibility (after several days of drying) from almost no effect at 1% to a very tacky situation at 50% and to an almost liquid condition at 75%. Seven percent was chosen as the concentration which offered maximum flexibility without undue tackiness. Tests with this 7% glycerol showed that the adhesion to the skin was excellent and that it retained its flexibility, as determined by comparison with straight PVP. The latter flaked off much sooner. The noise level was to some extent reduced as will be discussed in Section VI B. The flexibility data are summarized in table 4. It should be noted that the non-hygroscopic plasticizer, Aquaflex (1 part Aquaflex : 9 parts 25% PVP by weight) produced a very adequate degree of flexibility, even after driving off the water by prolonged heating and it was concluded that the

TABLE 4

Effect of various agents on plasticity of standard PVP strips. A brittleness grade of 0 represents the highest plasticity.

Additive	Concentration of Additive (per cent)	State of Strip	Brittleness Grade
Aquaflex	0	Heated to 80° C.,	3, 3, 3
	2	90 minutes and	3, 3, 3
	5	cooled	3, 3, 3
	10	" "	0, 0, 0
	15	" "	0, 0, 0
Triphenyl phosphate	10	Equilibrium with Atmosphere	3, 3, 3, 2, 2, 1 2, 1, 2, 1, 0, 1 (varies with humidity)
Isopropanol	75	"	0, 0, 0, 1, 0, 1
Isopropanol + 2.4 graphite: 1 PVP	75	"	1, 1, 0
n-Butanol	50	"	0, 0, 0
	74	"	0, 0, 0
	74	After heating to 80° C. 90 minutes	3, 3, 3
	74	After equilibrating with atmosphere	0, 0, 0
Isopropanol	70		1, 1, 1
Ethanol	70		2, 3, 2, 2, 2, 1, 1, 1
Glycerol	10		0, 0, 0
Isopropanol + 2.4 graphite (coarse) : 1 PVP	75		1, 1, 0
Isopropanol + 2.4 graphite (fine) : 1 PVP	75		2, 3, 3

mechanism by which this compound promoted plasticity was by the molecular slippage effect described by Mosier. This type of plasticizing is independent of ambient moisture and is therefore superior to the hygroscopic plasticizers. However, until toxicity tests are run on this compound, the use of the 7% glycerol mixture is deemed preferable.

C. CONDUCTIVITY OF HYGROSCOPIC MIXTURES

In converting the glycerol - PVP product to a conducting cement, the solution, containing 3 parts of 7% glycerol to 1 part of PVP by weight, was combined with graphite at the standard weight ratio of 2.4 gm. graphite (or 4.8 gm. silver) to 1 gm. of PVP dry weight. Unfortunately it was found that the conductivity of such mixtures was a variable matter. The variability seemed to be due to an increase in humidity with consequent swelling of the mass which could separate the continuous array of carbon granules into discreet, insulated particles. Conductivity was determined as a function of measured humidity in a variable humidity chamber. The graphite PVP samples were found to increase resistance, e.g. from 160 to 9000 ohms in a standard strip (3/8 of an inch spacing) when relative humidity changed from 67% to 87%. This effect was readily reproducible and reversible provided sufficient time was allowed for equilibration. Mixtures of silver and PVP in 7% glycerol exposed to the same range of humidity showed no change in resistance which remained at 1 to 2 ohms. The results on these tests are shown in figure 2. It is presumed that this effect is more a matter of particle size than of the characteristics of graphite versus those of silver. Since the degree of hydration of the strip when used on the skin would be affected not only by ambient humidity but also by the hydration of the underlying skin, it was feared that the use of graphite of the particle size available even in a fairly dry environment, might result in an insulated interface on the inner side of the solder layer. If silver was used as the conductor, it would have the added advantage of eliminating a junction between two different elements and with it the possibility of galvanic potentials or of contact potentials developing. Mixtures of silver, PVP, and glycerol, when spread on the skin, produced a flexible, tenaciously adhering cement and silver was adopted as the conductor of choice. Since the flexibilibility of the 7% glycerol sample was reduced somewhat by the addition of silver, mixtures containing 10% glycerol were tried, but with this concentration of glycerol, the tackiness of the cement became noticeable and its advantages over the 7% mixture did not justify its use.

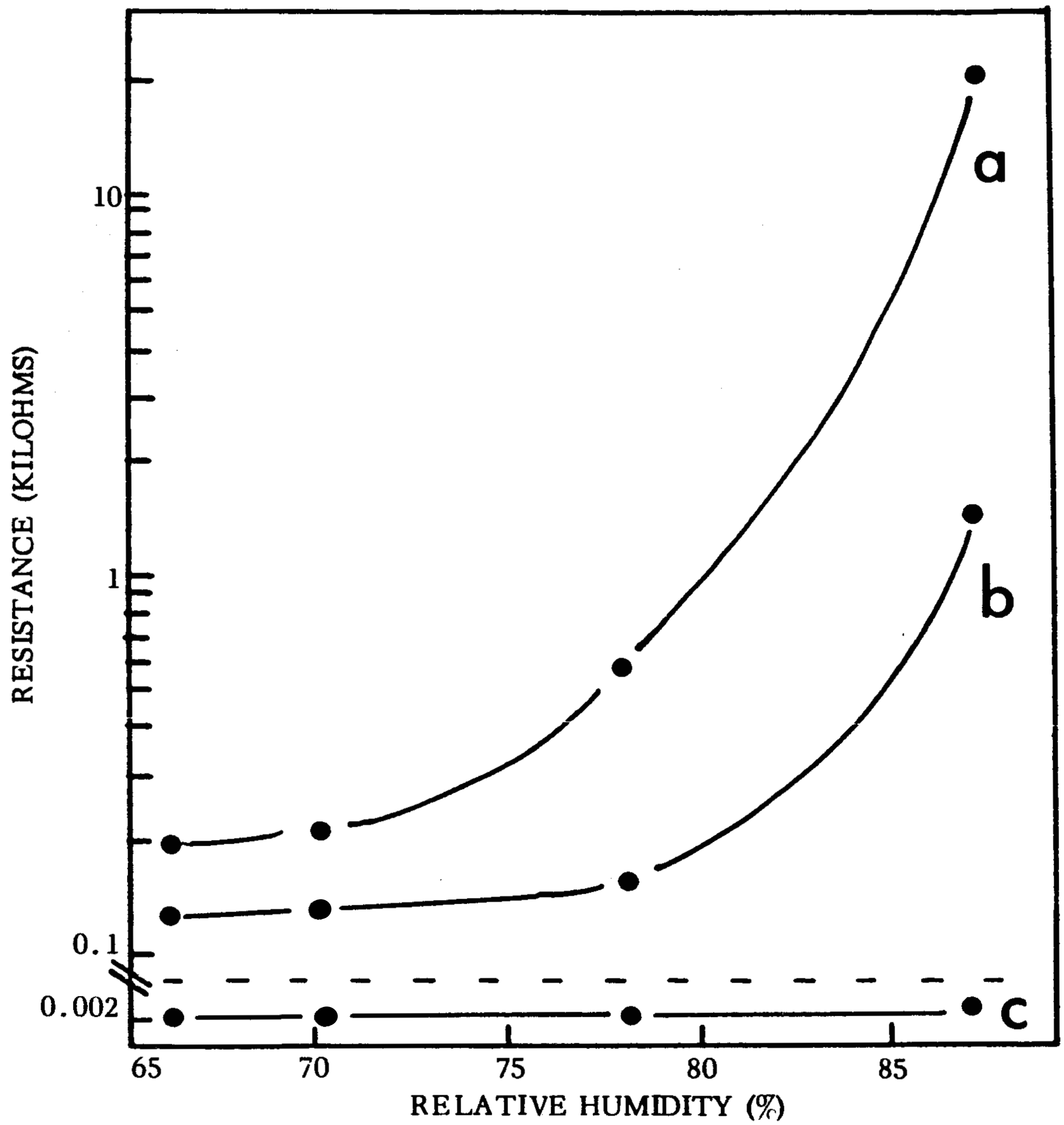

Figure 2. - Resistance of PVP strips (containing silver or graphite) as a function of humidity. Individual curves reproduced in 4 experiments on separate strips. Resistances taken with transverse embedded wire electrodes, 10 mm apart.

a. Graphite: PVP, 2.4:1, prepared in 7% glycerol.

b. Graphite: PVP, 2.4:1, prepared in water.

c. Silver: PVP, 4.8:1, prepared in 7% glycerol.

IV LONG TERM DETERIORATION OF CONDUCTIVITY OF AN INFILTRATED SKIN SITE

A. DETERMINATION OF MECHANISM

Despite the use of the optimum set of conditions for infiltrating the site and making electrical contact, the conductivity of the site continued to deteriorate over the course of time. Two clues appeared which gave some indication as to the possible cause of this deterioration. The first was the indication that sites covered with the PVP mixture retained their conductivity significantly longer than did those which were not covered (table 10). This beneficial effect of PVP could have been due either to its interference with mechanical disruption of the silver lattice or to effective screening out of the oxygen supply which may have been oxidizing the infiltrated mass. The second clue was the observation that strips of filter paper initially showing a very high conductivity, when stored in a drawer undisturbed for several months showed almost infinite resistance. If the cause of the deterioration is the same in both cases, a mechanical manipulation effect is largely discounted, and a chemical change is implicated as the causative factor, the most feasible one being the formation of the oxide or sulfide of silver by a combination with atmospheric components.

1. Method and Results

To test this supposition, fresh silver infiltrated strips were prepared and exposed either to tincture of iodine or to the hydrogen sulfide fumes produced by a concentrated solution of sodium sulfide. All experiments were run three separate times. In each case the resistance of the standard strip increased from an original value of about 100 ohms to the order of 50,000 ohms. Reduction of the iodide with developer restored the original low resistance. The oxide was produced artificially by anodizing freshly prepared strips in 0.1 M NaOH solution. The high resistance of the product could be readily reversed by cathodizing in 0.1 M NaCl solution. When the aged filter paper strips were subjected to this cathodizing process, their high resistance was reduced to the order of the original readings of four months previously. It is significant that while silver iodide was relatively easily reduced, the silver sulfide required overnight exposure to the reducing agent and that the silver oxide could not in any way be reduced except by the electrical process.

The possibility that the processing with electrolytes left a residuum which gave rise to the increase in conductivity was eliminated by thorough washing and complete drying and by control strips subjected to the same treatment without the passage of current. The various results are summarized in table 5. Filter paper strips which had been

TABLE 5

Effects of iodine, H_2S, oxidizing in NaOH and aging on resistance of dry silver-infiltrated filter paper, and the reversal of these effects by reduction or cathodizing. Each row represents an experiment in triplicate.

Resistance after Silver Infiltration	Exposure	Resistance after Exposure	Reversal Process	Resistance after Reversal Process
60 ohms	Tincture of Iodine - 3 min.	>10 K	X-ray developer	250 ohms
300 "	"	>10 K	"	170 "
50 "	"	250 K	"	55 "
20 "	H_2S - 30 min.	25 K	X-ray developer	Could not
15 "	"	100 K	"	reverse
150 "	Anodized in 0.1 M NaOH	50 K	Cathodized in 1.0 M KCl	25 K
70 "	"	100 K	"	4 K
350 "	Soaked in 0.1 M NaOH	380 K	Soaked in 1.0 M KCl	340 ohms
100-200 "	Aged 6 months	>10 meg.	Cathodized in 1.0 M NaCl	100 ohms

impregnated months earlier with molten paraffin showed no loss of their conductivity when the testing electrodes were pressed into the paraffin at each end of the impregnated portion. It was concluded from the above experiments that an oxide or halide formation is the most likely cause of the deterioration of conductivity occurring in the case of filter paper and probably in the skin experiments as well.

Added evidence for this extrapolation to skin was obtained when fresh pigskin (obtained from the slaughter house) was submitted to these same procedures. The pigskin proved to be difficult to infiltrate even when the fatty material was dissolved away in petroleum ether. The observations on filter paper suggested that this failure was due to premature binding of the silver by agents in the skin such that the subsequent exposure to the reducing agent failed to achieve reduction. On this basis the pigskin was pre-treated with ascorbic acid to inactivate the possible binding agents; this treatment proved successful in enabling highly conductive infiltrations. A typical comparison of resistances was 70 ohms with pretreatment, 50, 000 ohms without pretreatment. Exposure of the conductive pigskin to the sulfide or to iodine caused a great increase in its resistance. These results are summarized in table 6.

TABLE 6

Effect of pre-treatment with ascorbic acid on success of subsequent silver infiltration of pigskin. Effects of iodine and H_2S on resistance are shown. All samples were run in triplicate and all measurements are for dry strips.

Pretreatment	Resistance after infiltration with silver	Exposure	Resistance after exposure
None	$>$20 Megohms		--
Pre-soaking in 0. 5 M ascorbic acid - 20 minutes	120 ohms	Tincture of Iodine	$>$10 Meg.
" " "	70 ohms	H_2S - 24 hrs.	50 K

B. PROTECTIVE PROCEDURES

Three methods for preventing this oxidative deterioration of a silver infiltration present themselves, namely, (a) to leave a residuum of reducing material with the infiltrated mass to act as a protecting agent, (b) to substitute for the silver a non-oxidizable material, such as gold, and (c) to physically isolate the silver network from the oxidizing material by means of a non-aqueous imbedding material. These approaches were tried on filter paper strips, on pigskin, and on humans.

1. In vitro

Filter paper and pigskin were freshly infiltrated with silver and their resistances measured. In the first method they were dipped in a solution of 0. 5 M ascorbic acid for a few minutes, removed, and air dryed. In a second case the infiltrated strip was made the cathode in a bath of 0. 5 M $AuCl_3$, the anode being silver, and exposed to a current of 6 ma. for 10 minutes. This gold plating method was attempted after it was determined that the substitution of gold chloride for silver nitrate in a conventional processing of a filter paper strip did not produce a conducting network except at isolated points where for some reason the gold was heavily laid down. However, in most parts of the strip whether on filter paper or pigskin, the gold infiltration alone did not produce a conductive network. In a third method, the infiltrated strips were impregnated with mineral oil or molten paraffin except at the very tips where electrical contact was made. These strips along with control strips not subjected to the protective measure were suspended over sodium sulfide solutions so as to be exposed (except for the ends) to hydrogen sulfide fumes. Although the conductivity changes in the controls took place within a half hour, the exposure to hydrogen sulfide was continued overnight and in each case full protection was afforded by the approach used. It is of special interest that cathodizing in NaCl protected the strips against the effect of H_2S. Each test was run in triplicate; results are summarized in table 7.

2. Protective Treatment on Humans

A limited number of experiments to test the efficacy of these procedures in preventing deterioration of the site has been run on human subjects. Pre-treatment with ascorbic acid for 20 minutes, although not successful in all subjects tested, was of some advantage (table 8) and it seems advisable to incorporate it in the procedure if time permits. As in the case of filter paper and pigskin, infiltration of human skin with gold alone did not produce a conductive dry site; however, it was possible to gold-plate an infiltrated site using the following method: the site was exposed to a puddle of 0.5 M $AuCl_3$, a silver anode was dipped into this puddle and two fingers of the hand were dipped into a solution of saline in which a silver cathode was immersed; the battery supply was 1.5 volts, applied for 10 minutes. In this way the silver reticulum in the corneum

TABLE 7

Effect of various protective procedures against the action of iodine, H_2S, or aging. Each test shown was run in triplicate. The protective effect of cathodizing is reduced if strips are washed prior to test exposure. All resistances are of dry strips.

Material	Protective Process	R_1	Exposure	R_2
Filter paper	None	150 Ohms	Anodized in 0.1 M NaOH	50 K
	Infiltrated with paraffin	70 "	" " "	2.5 K
	None	100-150 "	Aged 6 months	>10 Meg.
	Infiltrated with paraffin	20- 30 "	" " "	20 Ohms
	None	50 Ohms	Tincture of Iodine	250 K
	Infiltrated with mineral oil	50 "	" " "	60 Ohms
	None	36 Ohms	Aged 27 days	300 K
	Wet with 0.5 M ascorbic acid and dried	26 "	" "	70 Ohms
	None	25 Ohms	Aged 36 days	>1 Meg.
	Cathodized in 0.5 M $HAuCl_4$ (not washed)	40 "	" "	60 Ohms
	None	25 Ohms	H_2S - 90 min.; washed	>10 Meg.
	Cathodized in 0.5 M $HAuCl_4$ (not washed)	20 "	" "	80-1500 Ohms
	Cathodized in 0.5 M NaCl (not washed	20 "	" "	45 Ohms

TABLE 7 (cont'd)

Material	Protective Process	R_1	Exposure	R_2
Pigskin	None	120 K	Tincture of Iodine 30 minutes (washed)	>10 Meg.
	Cathodized in 0.5 M $HAuCl_4$ (not washed)	60 K	" " "	60 K
	Cathodized in 0.5 M NaCl (not washed)	1 Ohm	" " "	1 Ohm
	None	30 Ohms	H_2S - overnight	250 K
	Wet with 0.5 M ascorbic acid and dried	30 "	" " "	30 Ohms
	None	80 K	H_2S overnight	900 K
	Cathodized in 0.5 M $HAuCl_4$	60 K	" " "	60 K

TABLE 8

Influence of pre-soaking of human skin in ascorbic acid upon success of infiltration. Letters refer to different subjects. Resistances are for dry areas, 1 cm^2.

Concentration of Ascorbic Acid	Resistance (Kilohms)		
	A	B	C
0	137	73	123
0.1	140	50	140

Concentration of Ascorbic Acid	D	E	F	G	H	I
0	>300	273	40	40	174	83
0.5	5	160	33	27	110	153

became a cathode and was plated with gold as shown by the change in color. The resulting product was as conductive as the original silver site, but after one day showed a greater rise than did the control site. A summary of the results of these two protective procedures in humans is presented in table 9. It may be of special interest at this point to mention that for the sake of a thorough control procedure the infiltrated control site was also exposed to this cathodizing process, but with sodium chloride substituted for the gold chloride. The determination of whether the greater rise in resistance in the case of the gold plated site as compared to this sodium cathodized site was due to a deleterious effect of gold or a beneficial effect of cathodizing in NaCl (e.g. by reduction of formed oxides) remains to be determined in future experiments. The approach to the third method of protection, that is, physical isolation in the human, has not been adequately tested to date. However, as pointed out in the previous section, covering of the site with PVP which presumably restricts the atmospheric oxygen or sulfide supply also enhances the durability of the site (table 10). This, coupled with the favorable results achieved with filter paper and pigskin, make this a promising avenue of attack. The method of choice would likely be the exposure of the skin to a solution of a waxy substance in a volatile solvent to allow penetration by diffusion and then solidification upon loss of the solvent.

TABLE 9

Effect of various protective procedures in prolonging low resistance of silver-infiltrated human sites; three selected subjects out of eleven showing variability in success. Areas are 0.31 cm^2, resistances in kilohms. Each row represents a separate site.

		TIME (DAYS)		
Subject	Treatment	0	1	2
A	None; left uncovered	200	800	>1000
		23	550	900
A	Covered with PVP-	300	150	300
	graphite	100	100	450
		150	90	150
A	Wet with 0.5 M	190	100	400
	ascorbic acid and	250	90	250
	dried;			
	Covered with PVP-	25	80	150
	graphite			
B	Soaked in 0.5 M	75	550	
	$HAuCl_4$	70	400	
B	Cathodized in 0.5 M	50	100	
	$HAuCl_4$	30	130	
B	Cathodized in 0.5 M	55	80	
	NaCl	50	100	
C	Soaked in 0.5 M	90	600	
	$HAuCl_4$	70	600	
C	Cathodized in 0.5 M	80	500	
	$HAuCl_4$	70	700	
C	Cathodized in 0.5 M	60	100	
	NaCl	55	120	

V FORMATION OF A REVERSIBLE SYSTEM

Many of the anticipated applications of a long term electrode would entail AC measurements only, and for this purpose the simple infiltrated silver or gold-plated site would suffice. In cases where either DC potentials were being recorded or where a current was being introduced through the electrode, it would be highly desirable that the system be reversible. Efforts were consequently directed toward converting the infiltrated silver site to a stable silver-silver chloride system.

Although silver chloride can be formed by exposure of silver to hydrochloric acid for a long period, the application to human skin essentially calls for anodizing as the method of choice. Experiments in this area were notoriously unsuccessful and never got beyond the filter paper stage. In these experiments the infiltrated strip was made the anode in a solution of KCl which was varied between 0.1 M and 1 M, with a silver metallic strip as the cathode. Currents of varying density were passed through these strips for varying durations and with various schedules of alternation of current direction, all with essentially the same result. When the strip which had been freshly "anodized" was measured in combination with conventionally formed silver-silver chloride electrode or in combination with another freshly anodized filter paper strip, the measured potential would fluctuate over a range of as much as a half a volt; it might gradually go from 500 millivolts to 0 and then beyond, generally in a relatively unpredictable manner. The most stable preparations measured were those which had been anodized at all. Clearly some characteristic of the deposited silver in the finely divided state was causing a dramatic change in its behavior after the anodizing process.

Despite the complete lack of promise of this attempt, the use of this electrode for DC measurements or handling of currents may be adequate even in the raw metallic state because of the large surface area. Thus it would behave something like platinized platinum (platinum black). The bias potentials between any two of these filter paper strips when fresh were uniformly low generally less than 1 mv. Furthermore, the behavior of infiltrated skin sites, when used in the measurement of skin resistances with a current of 8 microamps/cm^2 was the same as that of the conventional wet silver-silver chloride site. Although the unfavorable results of this aspect of the study may be attributed to the presence of cellulose in an intimate relation with the infiltrated silver, it should be noted that results with leather were essentially similar.

VI TESTING THE SYSTEM ON HUMAN SUBJECTS

A. EVALUATION OF CONDUCTIVITY EFFECT

The examination of the effect of various procedures at various stages of this investigation required observations on human subjects. As the system became more efficiently developed, variations in procedure were logically introduced and new tests made with the new procedure. Anticipating this constant development and modification of the method, at no time to date has an exhaustive evaluation of the overall procedure been made on a large population because of the probability that future findings would call for new surveys of the populations. Consequently the human evaluations consist of an assortment of tests run at various stages in the development, usually no more than four to six subjects at a given stage, though occasionally more. In general the experiments at any given stage were carried out until a useful indication was obtained of the effect of the given parameter under observation but the exhaustive tests were delayed until arrival at the final total method of choice. Thus the evaluation gives a reflection of the effect of each of the parameters considered and gives some indication of the anticipated degree of reproducibility, durability, resistance, and noise level, but it is not represented as a valid reflection of the population as a whole.

1. Method

The effect of various parameters has already been referred to in part in tables 2, 3, 4 and 10. In addition to those experiments, a series of extended observations were made on human subjects who were infiltrated with silver by the method described in Section II-E, covered with skin solders of various compositions with regard to silver, graphite, and glycerol content and observed over a period of from three to eight days. In these experiments it was necessary to cover the PVP with waterproof coating so that extended observations would not be invalidated by accidental wetting of the site. It was found that the adhesion of Pliobond to the skin and to PVP was excellent for several days, but that the Pliobond would eventually loosen from the skin. However, an underlying coating of tincture of benzoin which is a mixture of balsam resins, made an excellent cement bond between the skin and the Pliobond and also protected the skin against the possibly injurious effect of the methyl ketone solvent used in the Pliobond. Unfortunately it was later found out that individuals may be sensitive to tincture of benzoin, producing a severe inflammatory response (one subject out of thirty as validated by later patch tests at the suggestion of Dr. John Knox, Baylor University College of Medicine). It is suggested that if tincture of benzoin is used to enhance adhesion to the skin a patch test should be run if at all possible, at least three or four days prior to its use in an infiltration.

2. Results

The results in table 10 summarize the findings on these experiments. This table shows resistance as a function of the parameter varied and of the age of the prepatation. Wet sites have been used as controls in many cases and serve as an indication of the resistance of the living barrier layer. A complete infiltration of the dry corneum should allow resistances as low as those of the wet control sites. This proves to be the case under many conditions.

It should be noted that the subjects bathed and wet the outside of the Pliobond-protected sites without any apparent deleterious effect. An inflammatory response would occasionally occur. In one case an inflammation occurred at the edge of one of the sites where a small eruption had been noticed prior to the infiltration.

B. EVALUATION OF NOISE LEVEL

Noise levels were tested on many of these same preparations, especially after completing the infiltration and covering with PVP. These measurements were made under two conditions: (a) at intermediate gain when a current of 8 microamps/cm^2 was traversing the preparation and (b) at very high gains when no external current was imposed. Noise levels were expressed not in terms of total white noise, but rather in terms of the amplitude within various frequency bands as determined by

TABLE 10

Effect of various skin-solder parameters on durability of sites, 0.31 cm^2.

A. Effect of covering.

B. Comparison of graphite and silver, each in 7% glycerol-PVP and of silver in varying concentrations of glycerol. Differences are not statistically significant.

A

	Resistance (Megohms) Initial	24 hrs.	Initial	24 hrs.
No covering	.55	7.0	1.10	4.0
PVP covering	.30	.16	1.20	.28
No covering	1.00	8.0	.70	8.0
Paraffin covering	.90	.18	.55	1.3

B

Composition	Graphite-PVP in 7% Glycerol	Silver-PVP in 7% Glycerol	Silver-PVP in 10% Glycerol	Silver-PVP in 15% Glycerol
N	3	4	4	4
Average ratio of resistance to that of starch control on day 5-6	2.47	1.88	2.06	1.84

TABLE 10 (C)

Effect of skin solder preparation on durability of human silver-infiltrated sites. Comparison of graphite and silver, each in 7% glycerol-PVP and effect of undercoating with tincture of benzoin. Subjects are different from those in 10 B. Graphite produced higher resistances than silver in 7 out of 10 cases (combining tables B and C); the remaining 3 were equal.

Composition	Graphite-PVP in 7% Glycerol	Silver-PVP in 7% Glycerol	Silver-PVP in 7% Glycerol
Undercoat	None	None	Tincture of Benzoin
Average ratio of resistance to that of starch control for day 4-5 (N = 7)	2.04	1.67	1.47
Average ratio for day 6-7 (N = 4)	2.17	2.11	1.62

measurement from the paper write out. The frequency response of the Offner system used in these measurements was flat to 40 cps. A summary of the noise level data obtained on human subjects under a variety of conditions including the calibrated disturbance described in Section III-A is shown in table 11. These data indicate that the noise generated is primarily a function of the composition of the skin solder used, since the procedure for infiltration of the sites was uniform.

In the course of the study of noise levels efforts were made to investigate the effect of particle size. These data, too, are included in table 11. It is seen that the noise level becomes less as the particle size is reduced. This finding may represent a promising approach to the reduction of noise level to a level suitable for EEG measurements and further investigation along this line awaits only the availability of graphite or silver dispersions of smaller particle size. It should be noted, however, that there are some indications that as the particle size becomes smaller the conductivity of a given mass dispersed in the PVP may become less. The success of this approach would probably depend upon whether a suitable compromise system could be reached.

TABLE 11 (A)

Noise level (microvolts) of silver-infiltrated filter paper and leather under various contact conditions.

Condition	Spontaneous Noise 0.2-0.3 cps	Spontaneous Noise 10-20 cps	Artifact from Mechanical Disturbance
Two strips of silver-infiltrated filter paper in 0.05 M NaCl	2	<1	
Same for leather	1-2	<1	
Dry silver-infiltrated filter paper strips with PVP skin solder electrical contacts:			
Graphite - No glycerol	<1	<1	2-6
Graphite - 7% "	<1	<1	2-24
Graphite - 15% "	12	2	150-200
Silver - 7% glycerol	<1	<1	1
0.6 Graphite-1 PVP-7% glycerol	<1	<1	28-48
0.9 Graphite-1 PVP-7% glycerol	1-2	6	200-400
1.2 Graphite-1 PVP-7% glycerol	<1	<1	1-2

TABLE 11 (B)

Noise level of silver-infiltrated human skin with skin solder contacts of various compositions. Starch-saline wet sites with Ag/AgCl electrodes are shown for comparison. N = number of subjects.

Frequency Band- cps		Starch-NaCl (Wet)	Silver-PVP in Glycerol	Graphite-PVP in Glycerol	Graphite-PVP in H_2O
		Composition			
	N	9	11	10	11
0.2-0.3	Avg. Amplitude	10.0 µV	12.4 µV	10.0 µV	11.6 µV
	Median	4	12	7	10
	N	9	11	10	11
3-6	Avg. Amplitude	3.8	9.4	6.8	8.3
	Median	3	7	5	5
	N	9	10	10	11
10-30	Avg. Amplitude	3.9	4.0	3.3	4.3
	Median	3	3	3	3
Artifact from Mechanical Disturbance	N		1	1	1
	Amplitude		84	140	280

Frequency Band- cps		Silver-PVP in 7% Glycerol	Silver-PVP in 10% Glycerol	Silver-PVP in 15% Glycerol
0.2-0.3	Avg. Amplitude (N=3)	12.7 µV	18.3 µV	13.3 µV
3-6	Avg. Amplitude (N=3)	11.0	14.0	11.7
10-30	Avg. Amplitude (N=3)	4.7	3.3	4.0
	Artifact-Mechanical (N=3)	858	817	870
	Potential difference between adjacent similar sites (N=3)	1.29 mV	3.99 mV	6.86 mV

TABLE 11 (C)

Ratio of amplitudes of noise levels with skin solders containing fine graphite versus coarse graphite (four subjects).

Frequency Band- cps	Ratio of amplitudes (fine/coarse graphite) 1	2	3	4
0.2-0.3	.20	.50	.23	.87
3-6	.47	.40	.04	1.00
10-30	1.67	.60	.80	1.50

TABLE 11 (D)

Noise level of various preparations during the passage of 8 microamperes of current.

Preparation	Silver-infiltrated leather in 0.05 NaCl	Silver-infiltrated skin sites - 1 cm^2	Wet starch-NaCl skin sites
Noise with passage of 8 μAmp. current	8-10 μV	$<$0.2 mV (30 mV full scale)	$<$0.2 mV (30 mV full scale)

VII THE PROBLEM OF THE LIVING BARRIER LAYER

A. ANATOMICAL SURVEY OF SKIN RESISTANCE

During the development of the infiltration technique it was found that in numerous cases the resistance of the completely dried site equaled that of the wet site but that the resistance level of both were unacceptably high, for example, 200,000 ohms cm^2; this resistance represents that of the underlying cellular barrier.

This resistance of the living barrier layer is well known to be a function of the anatomical site (9) and especially of the state of the subject (4, 10) but the literature is very scantly on the range of expected values of any given site on the body. Since it is of paramount importance in line with the input requirements of the first stage of amplification to be aware of the expected resistance range at any intended site of measurement, a survey was made on a series of subjects to obtain samples of specific resistance of skin at various parts of the anatomy.

1. Method

Sites 1/4 inch in diameter, were masked off in various skin areas with a technique described elsewhere (1) and a starch saline paste applied as described in this same paper. A plate approximately 12 x 6 centimeters was used as a return electrode in the resistance measurements. Its large area, approximately 200 times that of the site being measured, insured that its contribution to the total resistance would be negligible. The sites were allowed to equilibrate for 15 minutes and the resistance data collected over as short a period as possible to minimize errors introduced by change of state of the subject.

2. Results

These data are summarized in table 12. They have been expressed in terms of the average resistance at a given site for N subjects and in terms of the average ratio of resistance of a given site to that of the palm. In this latter way a better picture of the resistance gradients along the body surface is obtained with the palm, which is normally one of the least resistive sites on the body acting as a reference having a value of 1. It will be noted that in addition to palm and sole there are several loci representing unusually low resistances, namely the hairy areas such as the scalp, the corona of the nipple, and the sternum. The data indicate that unless an artificial means of reducing the underlying resistance is achieved that many sections of the body will not be measurable with existing instrumentation even if the corneum is completely conducting.

B. EFFORTS TO REDUCE RESISTANCE OF UNDERLYING BARRIER

It has been pointed out elsewhere (2) that the barrier layer of cells which represents the primary component of the skin's resistance is

TABLE 12

Average skin resistances of N subjects for 1 cm^2 sites at various regions of the body surface.

Site	N	Average Resistance (Kilohms)	Average ratio of conductance at site to conductance of same subject's finger (palmar surface)
Finger - Palmar	19	79	1.00
- Dorsal	5	156	.43
Hand - Palmar	12	103	1.21
- Dorsal	5	385	.32
Wrist - Volar	11	335	.38
- Dorsal	5	288	.36
Forearm - Volar	19	351	.43
- Dorsal	3	206	.37
Upper arm - Medial	12	546	.16
- Mid-Lateral	5	440	.18
- Deltoid	5	264	.26
Shoulder - Ventral	9	316	.33
Neck - Ventral	8	197	.53
- Dorsal	6	164	.84
Forehead, midway between eye and scalp	5	56	1.55
Scalp (1-1/2 inches from mid line)			
- Coronal suture	7	23	4.41
- Parieto-occipital	7	46	2.96

TABLE 12 (cont'd)

Site	N	Average Resistance (Kilohms)	Average ratio of conductance at site to conductance of same subject's finger (palmar surface)
Chest - Lateral wall in mid-axillary line at level of 6th rib	9	384	.35
- Vental, 1 inch below nipple	10	308	.47
- Sternum at level of nipples	8	139	.87
Back - Interscapular area at the level of the inferior angle of the scapula	9	250	.64
Abdomen - Lateral	9	216	.57
- Vental, one inch below navel	8	188	.62
Thigh - Lateral	5	306	.28
Leg - Lower, lateral	6	264	.60
Foot - Dorsal	6	222	.90
- 1 inch below medial malleolus	6	170	.89
- Plantar	6	189	.57

superficially located, is accessible to various solutes which easily transit the corneum and is susceptible to the actions of various agents which can reduce its resistance. These earlier experiments were performed under conditions in which no consideration was given to long term effect. It is clear that exposure of the skin for a considerable period to an agent which reduces resistance significantly in acute experiments might cause a severe irritation. The requirements for an agent which would reduce the resistance of the skin over a long term period are: (a) that it be non-toxic and non-irritating; (b) that it reduce the resistance of the skin at least 50%; (c) that it is not rapidly absorbed or metabolized by the skin; (d) that it is chemically stable. A guide in the search for this compound was the knowledge that the resistance of a living layer is determined primarily by cellular membranes and that these membranes are susceptible to the action of polar-non-polar compounds such as detergents. Many detergents will penetrate a membrane and become an integral part of it, changing its properties profoundly (3). With this in mind, the possible application of various polar-non-polar dyestuffs and detergents, as well as a series of agents which could be expected to act by denaturing the surface membranes, for example, formalin, were investigated. Many of the promising dyes had to be eliminated without trial because they were either potentially carcinogenic or toxic in other ways but numerous detergents were tested. The agents used in these experiments include: NaCl (5M), formalin, methylene blue, gentian violet, scarlet R, acriflavine, soap, Hemosol (a detergent phosphate), and a group of ionic and non-ionic detergents as follows:

Zephiran	Sodium benzalkonium chloride
Triton X	Nonyl phenol + 10 moles ethylene oxide
NX-7	Nonyl phenol + 30 moles ethylene oxide
Arquad T 2 C-50. . .	Quarternary-tallow-2coco-ammonium chloride
Ethoduomeen T-40 . .	Tallow-ethoxylated duomeen (30 moles ethylene oxide)
Armac CD-5D	Coco amine acetate
Onyx Isothan DL . . .	Dilauryl-dimethyl ammonium bromide
Ethomeen C-25 . . .	Polyoxyethylene (15) coco amine
Duomeen CD-50 . . .	Coco 1-3 propylene diamine

1. Method

A surface chamber 1/4 inch in diameter was fastened to the skin with Stomaseal, sites being located on the lateral surface of the upper arm. The return site was a vessel of 0.05 M NaCl into which two fingers of the hand were dipped so that an area of at least 20 cm^2 was exposed to the solution. A 1 M KCl-agar salt-bridge made contact with this vessel and the other end dipped into a vessel which contained either sodium chloride or tap water according to the solvent used to dissolve the test compound. Silver-silver chloride electrodes were dipped into this second vessel and into the test chamber on the arm to make the measurements. In most cases a series of six sites were monitored at one time. They were exposed first to tap water or sodium chloride according to the nature of the subsequent diluent for a 15 minute control period. The liquids were subsequently removed from the chambers, the skin dabbed dry and the test solution applied. Solutions were replenished at 5 minute intervals. Resistances were measured at 15 minute and 30 minute intervals to determine the effect of these compounds.

2. Results

It was found that of all products tested only 10% formalin, 5% acriflavine, 2% soap solution, 2% onyx Isothan DL, saturated methylene blue and 5 M NaCl had a consistently appreciable effect. The data are summarized in table 13. An agent which showed no effect after two trials was discarded. Agents which showed an appreciable effect were repeated several times more. After selection of the most suitable agents (5M NaCl, 10% formalin, 2% soap and 5% acriflavine), long term observations over a period of three days were made to determine the lasting effects. In this second group of experiments, after contact with the site for a half hour the solvent was allowed to evaporate so that an excess of the material remained in the skin. A control site in contact with water was treated similarly. The chambers were then sealed so that an air pocket covered the site and were left undisturbed for three days.

TABLE 13

Effect of various agents on resistance of wet skin after 30 minutes exposure. Descriptions of agents are listed in the text. NaCl concentrations are 0.05 M except as otherwise noted.

Agent	Conc.	N	Average ratio of resistance at site exposed to agent to resistance at control site exposed to solvent only
Triton - X	1/2%	1	1.00
	1%	1	1.17
	2%	1	1.12
Arquad T2C-50	1/2%	1	.80
	1%	1	.91
	2%	1	.93
Onyx Isothan in H_2O	2%	3	.46*
" " in NaCl	2%	1	.84
Armac C. D. in NaCl	2%	1	.92
Duomeen CD-50	1%	1	.94
Ethomeen C-25 in NaCl	2%	1	.96
Ethoduomeen T-40 in NaCl	1%	1	1.03
	2%	1	1.04
NX-7 in NaCl	2%	1	1.05
Zephiran in H_2O	2%	7	.66*
in NaCl	2%	6	1.03
Soap Solution	2%	8	.39*
Hemosol	1%	4	.66*
NaCl (ref. - H_2O)	.007 M	1	.82
	.05 M	5	.75
	5 M	8	.05*

TABLE 13 (cont'd)

Agent	Conc.	N	Average ratio of resistance at site exposed to agent to resistance at control site exposed to solvent only
Formalin in NaCl	0.1%	1	.86
	10%	1	1.11
" in H_2O	10%	8	.30*
Methylene Blue in NaCl	Sat.	1	.86
in H_2O	Sat.	3	.39*
Gentian Violet in NaCl	Sat.	5	1.02
Scarlet R in acetone	Sat.	3	1.52
Acriflavin in H_2O	5%	5	.11*
	30%	1	.07*

*- Appreciable reduction.

At the end of this time, all seals were removed and a drop of tap water was placed on each site and resistances measured immediately and followed for 15 to 30 minutes. It was demonstrated without exception in a series of duplicate runs on each of three subjects that within three days, the original beneficial effects of these compounds on skin conductivity had been completely lost. An example, one of these subjects typical of the others is shown in table 14.

The approach used does not, of course, exhaust the possibilities for achieving lasting reduction of the resistance of the living barrier layer but at this stage it is believed that it does not present a promising method for achieving the necessary conductance levels.

C. EFFORTS TO REDUCE THE RESISTANCE OF THE LIVING BARRIER LAYER BY MECHANICAL MEANS (ANIMAL EXPERIMENTS)

The resistance of the underlying barrier layer could be eliminated alternatively by abrasion or by biological changes induced by procedures affecting the deeper layers of the skin. The successful reduction of resistance by abrasion long has been recognized and is utilized in the preparation of EEG and EKG electrodes, with ground glass commonly being used within the paste and rubbed into the skin to abrade the corneum and frequently the underlying living barrier layer. It was expected, however, that this successful reduction of resistance

TABLE 14

Test of capacity of various agents to maintain reduction of skin resistance for extended periods. Agents were allowed to evaporate and sites were kept dry and covered for 3 days. Last measurements were made 1 minute after re-wetting with tap water. Resistances are for area 1 cm^2.

Site	1	2	3	4	5
Resistance in tap water (kilohms)	550	403	430	740	258
Agent exposed to	H_2O	5 M NaCl	10% Formalin	2% Soap	5% Acriflavine
Resistance after 30 min. (wet)	410	6	65	65	16
Resistance after 3 days (wet)	145	84	210	308	178

would be reversed by regeneration of the injured tissue. Efforts were therefore made to utilize growth inhibitors together with abrasion in order to prolong the effects. These growth inhibitors are usually enzyme inhibitors by nature and interfere with the growth process by blocking certain of the enzymatic reactions necessary for cell division. The two tried were podophyllin and colchicine, both of them being agents which have been used therapeutically.

1. General Method

Because these procedures were potentially injurious, this portion of the study was conducted on rats. The ultimate technique was as follows. The rat was lightly anesthesized with CO_2 and rapidly fastened into a chamber of polystyrene which, together with Ace bandage wrapping reinforced with lead for stiffening, prevented undue movement. Only the nose and the back were exposed. The rat regained consciousness usually within a minute after reaching anesthetic level and showed no ill effects of this treatment. In a preliminary series of experiments in which nembutal anesthesia was used, there was a high degree of loss due to complications developing as a result of the daily anesthesia necessary to follow the day-to-day progress of the procedure. With the CO_2 method as used in this acute short exposure only to facilitate securing of the animal, survival rate was almost 100%. The hair was clipped from the back of the rat and adhesive masks were applied to delineate the area to be subjected to the experimental procedure. Insofar as these experiments were aimed at evaluating the effect on the barrier layer, it was important to eliminate the contribution of the corneum to the resistance and consequently wet sites were used in most cases, the method being that previously referred to (1).

2. Results

a. Abrasion alone: Abrasion of a site by the skin-drilling method of Shackel (11) was found to reduce resistance by about 80 to 90%. The rate of regeneration was of the order of 3 days for 50% recovery, 5 days for 100% recovery. It is of interest that this recovery rate is of the same order as the rate of increase of resistance of an EEG site which is prepared by strong abrasion and it is likely that the rise of resistance in the NASA experiments at Huntsville, Texas (6) represented this same regenerative process (7). The rat data are summarized in table 15.

b. Abrasion plus growth inhibitors: When growth inhibitors were applied, it was found that these agents at higher concentrations (e.g. 1/2% colchicine) were highly irritating and promoted scab formations, while at lower concentrations they frequently did not succeed in retarding the rate of regeneration as reflected in the resistance measures. Unfortunately efforts to achieve a compromise intermediate concentration were unsuccessful. These results are also summarized in table 15.

TABLE 15

Duration of effects of skin-drilling in lowering resistance of rat skin and the effect of growth inhibitors on the recovery rate.

Procedure	Resistance as per cent of <u>intact</u> control site	Days to recovery of resistance to 50% of control site	Days to 100% Recovery
Skin-drilling alone	15	1	4
	3	1	2
	18	2	5
	2	3	4

Procedure	Resistance as per cent of skin-drilled control site undergoing simultaneous recovery — DAYS										
	0	1	2	3	4	5	6	7	8	9	
Skin-drilling plus podophyllin	81	87					83				
	92			94						94	
	60				100				83		
Skin-drilling plus colchicine											
1/4%	100	100	20								(thick scab)
	92		30	1330							(scab)
	160		200		6	9	20	100			
	100		140	60			150				(scab)
	100		100					100			
1/2%	25					100					(inflamed)
	100	100	50								(scab)
	100		67	330							(inflamed)
	152			200	4	6	20	100			(scab on 3rd day)
	100		100	50			25				(scab on 5th day)

c. Procedures affecting deeper layers (tattooing and inspection): At the suggestion of various NASA personnel, reduction of the resistance of this layer was attempted by the introduction of powdered graphite or other foreign bodies into the underlying corium either by tattooing or hypodermic injection. In all cases an undamaged control site and a control NaCl tattoo or control injection of sodium chloride was used to evaluate the effect of the agent used.

Tattooing was attempted both with a commercially available animal tattooing device and by commonly used needle technique. The agents used were (a) commercial tattooing ink, (b) a slurry of graphite granules, (c) a slurry of graphite granules stabilized with the protective colloidal action of low (e.g. 5%) concentrations of PVP, (d) isotonic saline, (e) nothing. All of these procedures were successful in reducing the resistance to a small fraction of that of the undisturbed control site. However, tattooing without any agent was just as effective as tattooing with any of the agents tried and it was concluded that the successful effects are to be attributed only to penetration of the barrier layer. Regeneration of these sites occurred essentially at the same rate as that of the abraded sites described in "a". The results are illustrated in table 16. Intradermal injections of foreign bodies were also used in attempts to produce a prolonged reduction in barrier layer resistance. Those selected were (a) tattooing ink, (b) a graphite suspension,

TABLE 16

Effect of tattooing procedure on resistance of rat skin.

Tattooing Agent	Resistance as per cent of intact control site	Days of recovery of resistance to 50% of control site
Blank tattoo	6	8
	20	1
	18	2
Tattoo ink	33	3
	4	4
	3	>3
PVP and graphite	6	8
	17	2

(c) PVP solution, (d) 1% Zephiran, (e) isotonic saline, (f) blank puncture. These procedures reduced the resistance approximately to the same extent but, unlike the results with tattooing, there were several instances in which the resistance of the site remained low for a considerable period. This effect did not seem to be peculiar to the nature of the compound injected so long as it was not saline. Best effects were obtained with PVP. The addition of graphite may have been helpful. These results varied greatly from site to site despite efforts to control the depth and angle of injection. Since PVP has been shown to be innocuous even when used as a plasma expander and since there were no signs of inflammation at the sites of these injections, it is believed that this approach may represent the most promising of those tried and that an adequate technique for controlling the depth of injection might be worked out for the human. The results of these tests are summarized in table 17. It is seen that in several cases the resistance was maintained at a low value for 8 days, in two cases for as long as 15 days. There was no sign of infection at these sites and it is presumed that the reduction of resistance was a consequence of mechanical interference with normal processes in the area.

VIII DEVELOPMENT OF A LONG TERM WET SITE

Because the development of the dry electrode was characterized by successes in some areas, failures in others, it was considered

TABLE 17

Effect of intradermal injection of various agents on resistance of rat skin and half-life of the effect.

Agent Injected	Resistance after injection (per cent of intact control site	Days to recovery of resistance to 50% of control site	
Blank	15	4	
(Penetration	26	1	
without	40	4	
injection)	40	5	
	47	1	Avg. 3
NaCl (0. 1 M)	12	5	
	17	1	
	58	1	
	7	1	
	5	1	Avg. 1. 8
Zephiran (1%)	6	5	
	33	8	
	20	>8	
	40	>8	>Avg. 7
PVP (20%)	20	4	
	20	15	
	17	10	
	11	5	
	6	8	
	12	1	
	12	5	
	14	4	
	19	8	Avg. 6. 7
PVP-Graphite	43	3	
	9	11	
	6	5	
	15	5	
	7	18	Avg. 8. 4

desirable to undertake a simultaneous development of a long term wet site. The approach to the problem of developing a long term wet electrode has been premised on three requirements: (a) the contact medium must maintain adequate conductivity down to the living barrier layer, (b) the compatibility of the skin with this agent must be such that long term exposure will not result in any skin reaction, and (c) the agent must be stable and not subject to absorption by the tissues. Long term contact of the skin with a conventional wet medium results in maceration and a generally unhealthy reaction and it would appear that under optimum conditions the skin should be in contact with a range of vapor pressure as might be encountered in ambient air. This could be accomplished if the solution used as the contact medium had a vapor pressure equal to that of the atmosphere. If such a solution also contained adequate electrolyte (of a type compatible with the skin) to allow appreciable conductivity, it might fulfill the requirements described above.

1. Method

To determine the concentration of a given agent which would be in equilibrium with the atmosphere at any given humidity level, various concentrations were initially prepared, spread out in a thin layer on a plastic Petri dish and exposed to the ambient air. Weighings were made initially and frequently during the first and second days and finally daily until plateau levels were reached. Room humidities were measured at the same time and the plateau values used to determine the amount of water taken up or given off and the final concentrations reached. These values were found to agree with one another and were used as the basis for preparing an equilibrium mixture which at any given humidity level had a fixed value. They did, however, vary with humidity, either taking on or giving off water. Representative curves are shown in figure 3. In this way it was determined that a glycerol concentration of 78% was in equilibrium with the typical relative humidity of 65% at this laboratory. A polyethylene glycol (Carbowax 600, average molecular weight 600) used routinely as a constituent of skin ointments and long term applications was also tried and its 90% solution in water was found to be in equilibrium with the atmosphere.

Initial evaluations of these compounds on humans were made in an open system because the problem of providing dependable, non-injurious attachment of an adequate chamber to the skin for a period of 8 to 10 days constituted a significant problem in itself and it was deemed advisable not to hold up the evaluation of this wet electrode for the selection of a suitable adhesive. In the method used, palmar sites on two or more fingers were masked off. A site prepared by the starch-saline method previously referred to was used as the control reference. After one hour of resistance and GSR measurements on these sites to check viability, the reference site was rinsed and dried; the glycerol or

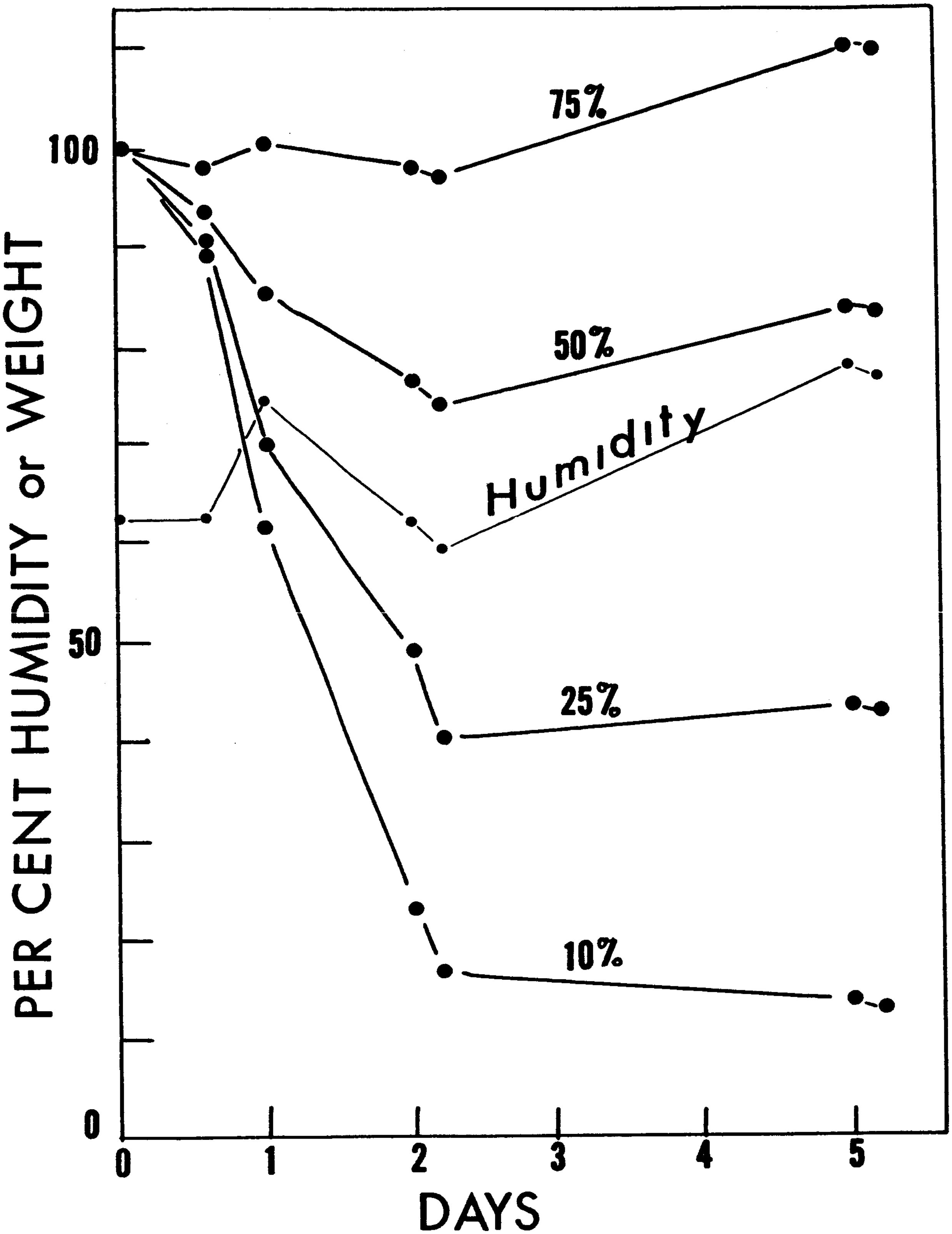

Figure 3. - Relative change in weight of glycerol solutions exposed to ambient humidity. Figures on curves are starting concentrations of glycerol. Solutions reach equilibrium at different times depending on initial moisture content.

Carbowax site was covered with a Band-Aid saturated with the test solution. The subject was given a small bottle of the test solution and was instructed to add more of this material to the Band-Aid four times daily. He was also given other Band-Aids and informed that should water accidentally wet the preparation he should remove the old Band-Aid, wipe the site dry, and apply a fresh Band-Aid strip saturated with the solution. He was also given rubber finger cots to cover the preparation during washing or bathing. When he returned for examination, usually on alternate days, a fresh reference site was prepared on the same area as previously. The experimental site was examined for signs of physical change and after half hour of stabilization of the reference site, readings of resistance and GSR were taken over a 15 minute period from both sites.

2. Results

Glycerol is a small, permeating, easily metabolized molecule and since initial tests showed no appreciable advantage over Carbowax, the long term tests were primarily limited to the Carbowax preparation. The resistance of the equilibrium mixture of Carbowax was appreciably higher than that of glycerol but calculations of the contribution of the corneum to total resistance showed that it falls within the requirements of the system. Evidence for this is seen in the comparison of resistances of a saline site with that of an equilibrium glycerol and an equilibrium

Carbowax site shown in table 18. Examination of the long term Carbowax sites revealed a healthy appearance and good GSR activity after 12 to 14 days of exposure. The resistance levels ran somewhat higher than those with a saline starch site, but this effect is not great enough to be considered an essential disadvantage of the method. It is especially noteworthy that in the event long term skin resistance and GSR recordings are to be made, this method would allow continuing viability of the site as determined by comparison of GSR amplitudes with those from the freshly prepared reference site. The results of these long term experiments on Carbowax are summarized in table 19.

IX SUMMARY OF THE SUB-CONTRACTOR'S EFFORT

The primary aim of the sub-contract with the Institute of Research and Instrumentation was a support study utilizing physico-chemical techniques that would lead to a better understanding of the nature of the ultra-structure of the silver infiltrated skin and the effect of the various variables on this ultra-structure. It was found initially desirable, however, to utilize the special capabilities of the sub-contractor in dealing with immediately pressing problems facing the Bio-instrumentation Branch at that Manned Spacecraft Center. Thus a considerable portion of this effort, approximately 40%, was spent

TABLE 18

Resistances and GSR amplitudes of human skin in contact with various media in comparison with a conventional medium of 0.05 NaCl in starch paste. Values are taken after 60 minute exposure to media and are ratios of resistances or GSR amplitudes, experimental/starch.

Medium	3% Natrosol 2% PVP in 0.1 M NaCl	78% Glycerol 0.05 M NaCl	90% Carbowax 0.1 M NaCl	3% Natrosol 2% PVP in 0.1 M NaCl	78% Glycerol 0.05 M NaCl	90% Carbowax 0.1 M NaCl
Subject	Resistance Ratio			GSR Amplitude Ratio		
1	.79		1.27	1.96		1.46
2	.58		.68	2.23		2.16
3	1.03		.44	1.22		.98
4	.91		.94	1.31		1.06
5	.54		.96	.83		1.90
6	1.23		1.56	1.40		1.74
7	5.06		3.82	2.68		1.72
8	-		.99	1.20		.89
9			1.37			1.80
10	1.25	.50	.96	2.86	1.10	1.52
11	1.45	2.12	1.31	1.31	1.15	1.15
12		.95			5.40	
13		1.98			1.08	
14		.85			3.12	
15		.60			1.85	

TABLE 19

Ratio of skin resistance and of GSR amplitude of a Carbowax site to that of a freshly prepared starch-saline site after long term continuous exposure to the Carbowax medium.

Subject	Days Exposure	Resistance Ratio First Day	Resistance Ratio Last Day	GSR Ratio First Day	GSR Ratio Last Day
1	14	1.20	2.30	2.40	.50
2	4	.92	1.30	1.01	1.35
3	13	.68	1.85	2.16	1.03
4	12	.58	2.25	1.15	1.33
5	12	.95	3.30	.80	.38
6	12	.96	.80	1.90	1.25
7	4	.99	2.05	.89	2.22
8	14	1.00	1.35	1.88	1.62
9	9	1.37	1.54	1.60	.96
10	14	3.48	4.66	1.25	1.44

in efforts (a) to improve the electrode paste used in the Mercury flights and (b) to improve the stability and reversibility characteristics of the electrodes used in the Mercury series and probably for the Gemini and Apollo series. A summary of the results of this effort is presented in the sub-contractor's report submitted separately. The portion of the sub-contractor's effort relevant to the dry electrode development will be briefly summarized here and treated in detail in his report.

A. CONDUCTIOMETRIC EXPERIMENTS

An indication of the uptake of silver by protein can be obtained by measurement of conductivity following addition of small increments of a silver salt to the solution which is in contact with the receiving protein. Initial increments of silver salt ($AgNO_3$) added to a gelatin solution will be almost entirely bound by the protein molecule and little increase in conductivity will occur. As the available sites become saturated with silver, additional increments will remain in solution and contribute significantly to an increase in conductivity. Thus if the current flow between a pair of standard electrodes in the solution is measured continuously as a function of the increment of silver added, the current will rise sharply beyond a certain amount of silver giving an indication of the saturation point of the system. In this way the effect of pH, salt concentration, and other variables upon the amount of uptake of silver by a given amount of protein may be ascertained. The relationship between pH, isoelectric point, and the amount of uptake of silver was such that it could be concluded the bonding of the silver ion is not at carboxyl or other acidic groups, but rather at active loci at which van der Waals forces allow adsorption.

Similar experiments were conducted in a specially constructed chamber in which two 1.3 cm^2 of pigskin were stretched across one end of a chamber and the conductivity measurements made in the solution in contact with this skin. Fresh pigskin, similar to that used in the experiments reported in another section, was used in these experiments. Silver was taken up by the pigskin optimally at pH 4.8 and pH 8.1, poorest at neutrality. The data indicated that silver ion, prior to its reduction, was bound to skin structures by the same adsorptive forces as was the case for the gelatin solutions rather than by a direct association with carboxyl groups. Variation of electrolyte concentration (KNO_3) from 0.1 to 1.0 did not appreciably affect uptake of silver.

B. ELECTRONMICROGRAPHIC EXAMINATION

The primary question which stimulated the electronmicrographic examinations in this study concerned the process causing the deterioration of the site over time since this was a primary weakness in the overall method. Consequently the samples chosen for examination consisted of freshly prepared samples of filter paper, pigskin, and human skin which showed good conductivity and in comparison, samples of these same specimens which had been made non-conductive either by aging or by exposure to hydrogen sulfide. In addition, samples which were processed in the usual manner but which showed some areas of good conductivity and others of poor conductivity were also examined, samples being taken from these respective areas. Finally pieces of pigskin which were pre-treated with ascorbic acid to prevent premature oxidation were compared with those in which the pre-treatment was omitted. Potting of the samples in epoxy resin in preparation for microtome sections was accomplished with the kind cooperation and advice of Dr. F. Gonzales of the Department of Experimental Biology at Baylor University College of Medicine. In general there was little difference in the configuration of the array of silver particles from one condition to another, but samples which were conductive showed a filamentous structure while non-conductive samples from the same specimen showed a granular structure. Another significant finding was the indication of oxide formation in the aged, non-conducting infiltration on human skin, as evidenced by the appearance of birefringence. There was no evidence of a change in configuration. This finding is compatible with conclusions reached independently regarding the mechanism of deterioration (Section IV).

X CONCLUSIONS

This report demonstrates that it is feasible as a practical procedure to bridge the high resistance of the dry corneum by converting it to a metallic conductor, and that one can make a low noise, dependable electrical contact with this site with the use of the newly developed skin aolder. A body of evidence has been gathered to demonstrate that the deterioration which has contributed to the variability in the permanence of these preparations on the human is due not to any physical rearrangement but rather to an oxidative process. Results to date on the use of protective measures show significant promise of success and it is felt that the problem of bridging the corneum in a lasting manner can be dealt with. The possibility of producing a lasting, appreciable reduction of the resistance of the living barrier with chemicals does not seem great. More promising is the further development of the use of an intradermal injection of PVP. If the use of an injection of PVP can adequately deal with this problem of barrier layer resistance the overall procedure is regarded as useful for practical application. If reduction of the living barrier layer cannot be achieved, it is recommended that the infiltration method be used on a sufficiently large skin area so that the resistance achieved is within the limits and the capabilities of the amplifier to be used. The noise level of the system, even at the present stage of development, is well below that necessary for most recordings such as EKG (fig. 4) and there are indications that it may be reduced to the 2 microvolts found commonly with wet electrodes and deemed necessary for adequate EEG recording.

The development of the long term wet site is adequate for recordings in which the viability of the skin is of utmost importance, that is, for GSR, but for other applications this method has the same problem with regard to the resistance of the living barrier layer as does the dry electrode. If an adequate means of attaching a chamber to the skin for long term operation is found, this method would also meet the requirements of the long term electrode except for the resistance of the underlying barrier. The dry electrode has the advantage that the skin solder represents a much more compact means of making the electrical contact, presents no leakage problem and is less irritating to the skin than are the commonly available waterproof adhesives necessary for sealing an aqueous chamber. The wet electrode has a pronounced advantage in regard to speed of application.

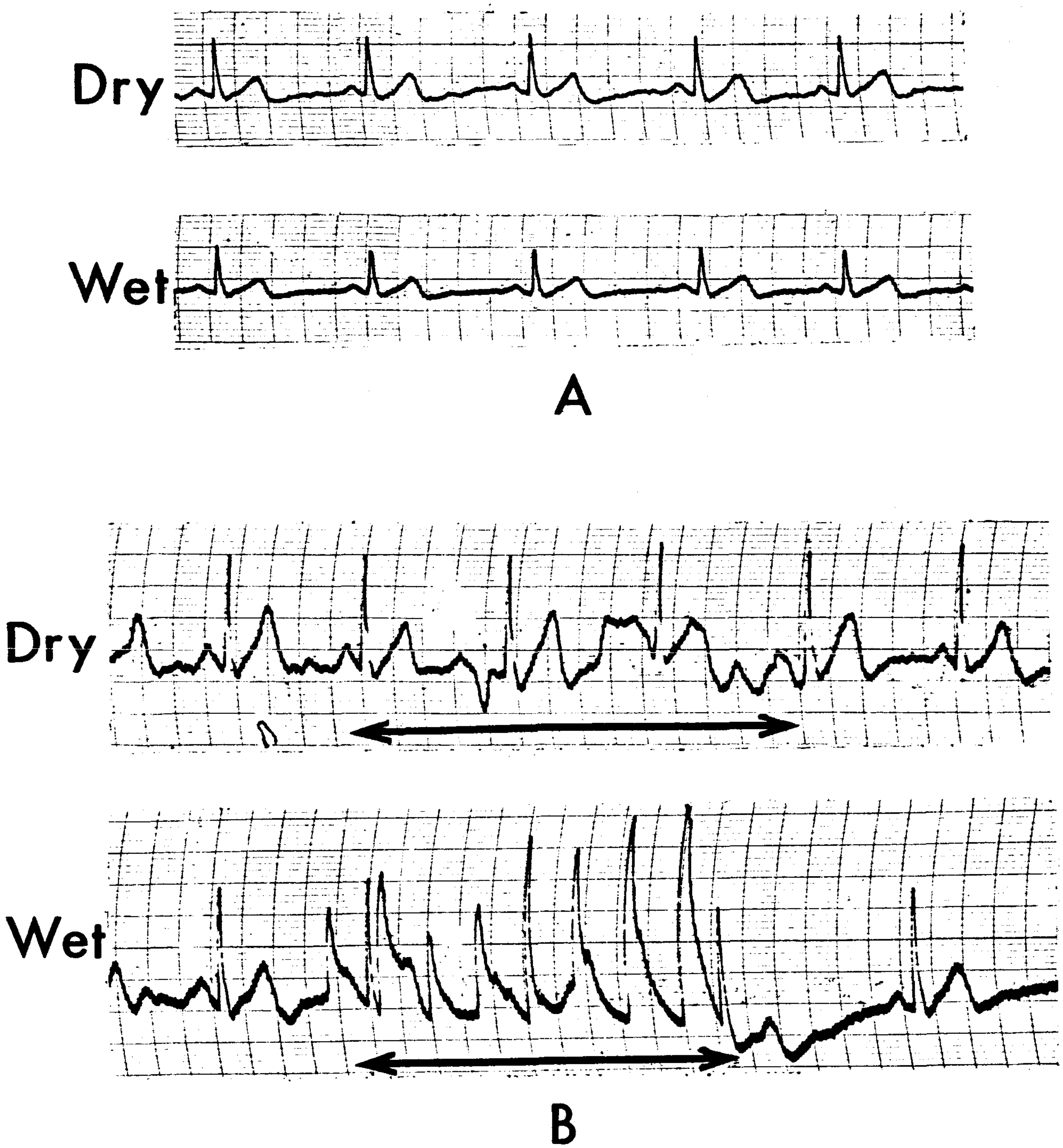

Figure 4. - Recording of EKG (lead I) with dry sites and conventional wet sites respectively.

A. Simultaneous recordings, sites undisturbed, same gain.

B. Sequential recordings; sites were tapped repeatedly during period above double arrow; same gain.

Contact on both A and B made with non-plasticized PVP-graphite skin solder.

XI RECOMMENDATIONS

The following recommendations are made:

1. That the method of choice for infiltrating and protecting against oxidation, incorporating all of the information obtainable from this study, be standardized and a population survey made to determine its long term dependability in terms of conductance, noise level, electrode bias and compatibility with the skin.

2. That efforts be continued on animals and humans to investigate the utility of intra- or subdermal injection of innocuous agents to reduce the barrier layer resistance.

3. That simultaneous efforts be devoted to the development of a method for fastening an aqueous chamber to the skin for long periods.

4. That efforts should be made to further reduce the noise level of the preparation so as to fall within the EEG range if the desirability of the dry site method for EEG application is sufficient.

5. That the infiltration procedure be examined from the standpoint of increasing speed of application.

6. That attention be given to the use of conducting Velcro cloth with PVP "skin solder" to make a convenient, comfortable electrical contact with the site.

7. That definite answers to partially investigated questions arising in the course of this study be found. These are:

a. Does pretreatment of a site with ascorbic acid significantly enhance chances for a successful infiltration?

b. Does cathodizing a site in NaCl after infiltration improve conductivity?

c. Does gold plating improve the permanence of the dry site?

d. Can impregnation with a wax-like substance improve the permanence of a dry site on human skin?

e. Does the chloride ion serve to act as a silver-binding agent which interferes with subsequent reduction?

f. Can the noise level of the preparation be reduced by using finer particles in the preparation of the skin solder?

g. Is the non-hygroscopic plasticizing agent, Aquaflex, non-toxic to humans?

h. Will the optimum pH condition for uptake of silver, indicated by the conductiometric studies, significantly improve infiltration?

XII SUMMARY

1. The feasibility of converting various materials including cellulose, leather, and human skin from a non-conductor to a good conductor by infiltration with silver salt and subsequent reduction has been demonstrated.

2. A method suitable for use on humans has been developed and found to produce skin resistances which are of the same order as those measured on a conventional wet electrolytic site.

3. A skin solder consisting of a suspension of conducting particles in a polyvinylpyrrolidone (PVP) matrix has been developed.

4. The noise level has been found to be a function of the nature of the electrical contact and has been reduced to 2 to 10 microvolts.

5. The deterioration of the high conductivity of silver infiltrated human skin has been shown to be due to a progressive oxidative process rather than to mechanical disruption of the conducting lattice and various protective measures have been shown to be effective in reducing this deterioration.

6. Even with complete bridging of the corneum, the conductivity of a site is limited by the living barrier layer to a level which frequently exceeds the maximum for the instrumentation presently available. A survey of the resistance of various anatomical sites shows that unless

this underlying resistance is artificially reduced, one can routinely expect an unacceptable resistance level if the present size of the electrode site is maintained.

7. Efforts to reduce the underlying barrier resistance by the use of abrasion and a growth inhibitor to prevent regeneration were unsuccessful as were efforts to reduce this resistance by continuous exposure to various chemical agents. The most successful procedure was the intra- or subdermal injection of a suspension of carbon or a solution of PVP in rats, but the method showed poor reproducibility because of failure to control the depth and angle of injection. In successful cases, however, resistance was kept at a very low level for as long as two weeks.

8. A suitable wet medium which could be applied to the skin for long periods, that is, two weeks, without showing any physical changes in the skin and without reducing the viability (as determined by GSR measurements) was developed on the principal that this medium should have the same vapor pressure as the ambient air. This condition was achieved by the use of high concentration of Carbowax (ethylene glycol) in an aqueous solution.

9. Conductiometric and electronmicrographic observations were made by the sub-contractor in an effort to shed light on the ultra-structure of the deposited mass of silver and the mechanisms governing successful and unsuccessful infiltration and deterioration. These observations

supported the independent finding that oxidation rather than physical rearrangement accounted for the deterioration in conductivity.

10. It is concluded that the method is a feasible one for practical application, provided further development of the intradermal injection technique is undertaken and provided the overall method of choice is evaluated on an adequate population of subjects.

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